



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>7</sup> : <b>C12N 9/10, 15/11, 15/54, 15/82, 7/00</b>		<b>A3</b>	(11) International Publication Number: <b>WO 00/32756</b>
			(43) International Publication Date: 8 June 2000 (08.06.00)
(21) International Application Number: PCT/US99/28354		(81) Designated States: AE, AL, AU, BA, BB, BG, BR, CA, CN, CR, CU, CZ, DM, EE, GD, GE, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 1 December 1999 (01.12.99)		Published With international search report.	
(30) Priority Data: 60/110,602 2 December 1998 (02.12.98) US 60/127,111 31 March 1999 (31.03.99) US			
(71) Applicant (for all designated States except US): E.I. DU PONT DE NEMOURS AND COMPANY [US/US]; 1007 Market Street, Wilmington, DE 19898 (US).		(88) Date of publication of the international search report: 12 October 2000 (12.10.00)	
(72) Inventors; and (75) Inventors/Applicants (for US only): CAHOON, Edgar, B. [US/US]; 2331 West 18th Street, Wilmington, DE 19806 (US). KINNEY, Anthony, J. [GB/US]; 609 Lore Avenue, Wilmington, DE 19809 (US). CAHOON, Rebecca, E. [US/US]; 2331 West 18th Street, Wilmington, DE 19806 (US).			
(74) Agent: FEULNER, Gregory, J.; E.I. du Pont de Nemours and Company, Legal Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).			

## (54) Title: SEQUENZES OF A PUTATIVE PLANT DIACYLGLYCEROL ACYLTRANSFERASES

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SEQ ID NO:25  MG-----DRGGA-----GSSRRRTGSRVS----VQGGSGPKVEEDEVDRDAVS
SEQ ID NO:26  MAILDSAGVTTVTENGGEFVDLRLRRRKSRS DSSNGLLLSGSDNNSPSDDVGAPADVR
SEQ ID NO:02  MAILDSAGVTTVTENGGEFVDLRLRRRKSRS DSSNGLLLSGSDNNSPSDDVGAPADVR
SEQ ID NO:08  -----
SEQ ID NO:14  MVGSDGDG-----DGGGGEAHAGGPRRRAGQ-----LRGLRDEAAPGSPRRPRPR
SEQ ID NO:16  MAISDEPESVATA-----LNHSSLRRRPSATSTAGLFNSPETTTDSSGDDLAKDSGSD
SEQ ID NO:22  MSKGNPDPLP-----GSFLPSHGGPPPKPTPRTFRNLPSSTHGFAPSVAAATIAT
1                                                    60

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SEQ ID NO:25  PDLGAGGDAPAPAPAPAHTRDKDGRTSVGDG-----YW---DLRCHRLQD
SEQ ID NO:26  DRIDSVVNDDAQGTANLAGDNNGGGDNNGGGGRGGEGRGNADATFTYRPSV-PAHRRARE
SEQ ID NO:02  DRIDSVVNDDAQGTANLAGDNNGGGDNNGGGGRGGEGRGNADATFTYRPSV-PAHRRARE
SEQ ID NO:08  -----
SEQ ID NO:14  PRPRG---GDSNGRSVLRPGG-----GGGRGGGGDFS---AFTFRAA-APVHRKAKE
SEQ ID NO:16  DSINS---DDAAVNSQQQNEK-----QDTDFSVLKFAIRPSV-PAHRKVKE
SEQ ID NO:22  TP-----PSASAPLPPTVHGEEAH---GAAAAARRD-----ALLPGVGAARRVKE
61                                                    120

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SEQ ID NO:25  SLFSSDSGFSNYR-GILNWCVVMLILSNARLFLENLIKYILVDP-IQVVSFLKDPYSW
SEQ ID NO:26  SPLSSDAIFKQSHAGLFNLCVVVLIIVNSRLIENLMKYGWLIRTDWFSSRSLRD---W
SEQ ID NO:02  SPLSSDAIFKQSHAGLFNLCVVVLIIVNSRLIENLMKYGWLIRTDWFSSRSLRD---W
SEQ ID NO:08  -----F---NATSLRD---W
SEQ ID NO:14  SPLSSDAIFKQSHAGLFNLCIVVLVAVNSRLIENLMKYGLLIRAGFWFNDKSLRD---W
SEQ ID NO:16  SPLSSDTIFRQSHAGLFNLCIVVLVAVNSRLIENLMKYGWLKSGFWFSSKSLRD---W
SEQ ID NO:22  SPLSSDAIFRQSHAGLLNLCIVVLIIVNSRLIENLMKYGLLIRAGFWFSARSLGD---W
121                                                    180

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## (57) Abstract

This invention relates to an isolated nucleic acid fragment encoding a diacylglycerol acyltransferase. The invention also relates to the construction of a chimeric gene encoding all or a portion of the diacylglycerol acyltransferase, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the diacylglycerol acyltransferase in a transformed host cell.

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# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference <b>BB1295 PCT</b>	<b>FOR FURTHER ACTION</b> see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. <b>PCT/US 99/ 28354</b>	International filing date (day/month/year) <b>01/12/1999</b>	(Earliest) Priority Date (day/month/year) <b>02/12/1998</b>
Applicant  <b>E. I. DU PONT DE NEMOURS AND COMPANY et al.</b>		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 8 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

**1. Basis of the report**

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☒ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

**SEQUENZES OF A PUTATIVE PLANT DIACYLGLYCEROL ACYLTRANSFERASES**

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☒ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

1  
☐ None of the figures.

# INTERNATIONAL SEARCH REPORT

ational application No.  
PCT/US 99/28354

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:  
1,3-12,15-25 all partially relating to SEQ ID NO 1, 2, 13-16, 21 and 22
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 3, 14 (complete) and 1,  
4-11 and 15-25 (all partially)

relating to SEQ ID NO:1,2

2. Claims: 1, 5-12 and 15-25 (all partially)

relating to SEQ ID NO:3,4

3. Claims: 1, 5-12 and 15-25 (all partially)

relating to SEQ ID NO:5,6

4. Claims: 1, 4-12 and 15-25 (all partially)

relating to SEQ ID NO:7,8

5. Claims: 1, 5-12 and 15-25 (all partially)

relating to SEQ ID NO:9,10

6. Claims: 1, 5-12 and 15-25 (all partially)

relating to SEQ ID NO:11,12

7. Claims: 1, 4-12 and 15-25 (all partially)

relating to SEQ ID NO:13,14

8. Claims: 1, 5-11 and 15-25 (all partially)

relating to SEQ ID NO:15,16

9. Claims: 1, 2, 5-11, 13 and 15-25 (all partially)

relating to SEQ ID NO:17,18

10. Claims: 1, 2, 4-13 and 15-25 (all partially)

relating to SEQ ID NO:19,20

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

11. Claims: 1, 4-12 and 15-25 (all partially)  
relating to SEQ ID NO:21,22



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 15/54, 15/82, 15/11, 9/10, 5/10, 1/21, C11B 1/00, A01H 5/00</b>	<b>A3</b>	<b>(11) International Publication Number:</b> <b>WO 99/63096</b> <b>(43) International Publication Date:</b> 9 December 1999 (09.12.99)
<b>(21) International Application Number:</b> PCT/US99/12541 <b>(22) International Filing Date:</b> 4 June 1999 (04.06.99)  <b>(30) Priority Data:</b> 60/088,143 5 June 1998 (05.06.98) US 60/108,389 12 November 1998 (12.11.98) US  <b>(71) Applicant (for all designated States except US):</b> CALGENE LLC [US/US]; 1920 Fifth Street, Davis, CA 95616 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> LASSNER, Michael, W. [US/US]; 721 Falcon Avenue, Davis, CA 95616 (US). RUEZINSKY, Diane, M. [US/US]; 849 Bouma Drive, Woodland, CA 95776 (US).  <b>(74) Agent:</b> EAGLE, Alissa, M.; Monsanto/G.D. Searle, Patent Dept. Central, P.O. Box 5110, Chicago, IL 60680-5110 (US).		<b>(81) Designated States:</b> CA, JP, MX, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>  <b>(88) Date of publication of the international search report:</b> 27 January 2000 (27.01.00)
<b>(54) Title:</b> ACYL COA:CHOLESTEROL ACYLTRANSFERASE RELATED NUCLEIC ACID SEQUENCES  <b>(57) Abstract</b>  By this invention, novel nucleic acid sequences encoding for acyl-CoA:cholesterol acyltransferase (ACAT) related proteins are provided, wherein said ACAT-like protein is active in the formation of a sterol ester and/or triacylglycerol from a fatty acyl-CoA and sterol and/or diacylglycerol substrates. Also considered are amino acid and nucleic acid sequences obtainable from ACAT-like nucleic acid sequences and the use of such sequences to provide transgenic host cells capable of producing sterol esters and/or triacylglycerols.		

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## INTERNATIONAL SEARCH REPORT

national Application No

PCT/US 99/12541

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/54 C12N15/82 C12N15/11 C12N9/10 C12N5/10  
 C12N1/21 C11B1/00 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C11B A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 45439 A (STURLEY STEPHEN L ; UNIV COLUMBIA (US)) 4 December 1997 (1997-12-04)  the whole document	1,7,8, 10,14, 25,28, 29,35
X	WO 94 09126 A (DARTMOUTH COLLEGE) 28 April 1994 (1994-04-28) the whole document  -/-	1,7,8, 14,25,28

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

16 November 1999

Date of mailing of the international search report

30/11/1999

Name and mailing address of the ISA

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## INTERNATIONAL SEARCH REPORT

National Application No

PCT/US 99/12541

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>YANG, HONGYUAN ET AL: "Functional expression of a cDNA to human acyl - coenzyme A: cholesterol acyltransferase in yeast: Species dependent substrate specificity and inhibitor sensitivity." JOURNAL OF BIOLOGICAL CHEMISTRY, (1997) VOL. 272, NO. 7, PP. 3980-3985. , XP002122738 the whole document</p>	1,7,14, 16,25, 28,29
X	<p>YU, CHUNJIANG ET AL: "Molecular cloning and characterization of two isoforms of Saccharomyces cerevisiae acyl-CoA: Sterol acyltransferase." JOURNAL OF BIOLOGICAL CHEMISTRY, (1996) VOL. 271, NO. 39, PP. 24157-24163 , XP002094336 the whole document</p>	1,7,14, 16,25,28
X	<p>CASES S. ET AL.: "Cloning and expression of a candidate gene for diacylglycerol acyltransferase" FASEB JOURNAL, vol. 12, no. 5, 20 March 1998 (1998-03-20), page A814 XP002122739 abstract</p>	1,7,8, 14,15, 25,28,35
X	<p>MARRA M. ET AL.: "AC W10786" EMBL DATABASE, 29 April 1996 (1996-04-29), XP002122740 Heidelberg the whole document</p>	1,7,8, 11,14
X	<p>MARRA M. ET AL.: "AC AA457966" EMBL DATABASE, 7 June 1997 (1997-06-07), XP002122741 Heidelberg the whole document</p>	1,7,8, 11,14
X	<p>WILSON R. ET AL.: "AC 045245" EMBL DATABASE, 1 June 1998 (1998-06-01), XP002122742 Heidelberg the whole document</p>	1,7,8, 13,14
X	<p>WO 95 27791 A (CALGENE INC ; DAVIES HUW MAELOR (US); HAWKINS DEBORAH (US); NELSEN) 19 October 1995 (1995-10-19)</p>	37
A	<p>the whole document</p>	1-39

-/-

## INTERNATIONAL SEARCH REPORT

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZOU J. ET AL.: "Modification of seed oil content and acyl composition in the Brassicacea by expression of a yeast sn-2 acyltransferase gene" PLANT CELL, vol. 9, 1997, pages 909-923, XP002122743 the whole document	37-39
A	FRENTZEN M (REPRINT): "Acyltransferases from basic science to modified seed oils" FETT-LIPID, (MAY 1998) VOL. 100, NO. 4-5, PP. 161-166. PUBLISHER: VCH PUBLISHERS INC, 303 NW 12TH AVE, DEERFIELD BEACH, FL 33442-1788. ISSN: 0931-5985., XP002122744 RHEIN WESTFAL TH AACHEN, INST BIOL 1, WORRINGERWEG 1, D-52056 AACHEN, GERMANY (Reprint) the whole document	1-39
P,X	CASES S ET AL: "Identification of a gene encoding an acyl CoA: diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 OCT 27) 95 (22) 13018-23. , XP002122745 the whole document	1,7,8, 14,15
P,X	OELKERS P. ET AL.: "Characterization of two human genes encoding acyl conenzyme A: cholesterol acyltransferase-related enzymes" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 41, October 1998 (1998-10), pages 26765-26771, XP002122746 the whole document	1,7,8, 14,15
P,X	HOBBS D H ET AL: "Cloning of a cDNA encoding diacylglycerol acyltransferase from Arabidopsis thaliana and its functional expression." FEBS LETTERS, (1999 JUN 11) 452 (3) 145-9. , XP002122747 the whole document	1-4, 14-16, 25,28,35
P,X	WO 98 55631 A (CALGENE LLC) 10 December 1998 (1998-12-10)  the whole document	1,7,8, 14-25, 28-39

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/12541

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9745439	A	04-12-1997	AU 3225997 A	05-01-1998
WO 9409126	A	28-04-1994	US 5484727 A	16-01-1996
			AU 5356694 A	09-05-1994
			US 5968749 A	19-10-1999
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WO 9527791	A	19-10-1995	US 5563058 A	08-10-1996
			US 5824858 A	20-10-1998
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			CA 2186607 A	19-10-1995
			EP 0754232 A	22-01-1997
			JP 9511650 T	25-11-1999
			US 5968791 A	19-10-1999
WO 9855631	A	10-12-1998	NONE	

## INTERNATIONAL SEARCH REPORT

International Application No

US 99/28354

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N9/10 C12N15/11 C12N15/54 C12N15/82 C12N7/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE EMBL [Online]  AC0064579, 1 August 1998 (1998-08-01)  ROUNSLEY S.D ET AL.: "Putative Acyl-COA:  Cholesterol Acyltransferase"  XP002132307  see sequence</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	1,3-12, 14-25

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OELKERS P ET AL: "Characterization of two human genes encoding acyl conenzyme A: cholesterol acyltransferase-related enzymes" JOURNAL OF BIOLOGICAL CHEMISTRY,US,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 273, no. 41, 9 October 1998 (1998-10-09), pages 26765-26771-26771, XP002122746 ISSN: 0021-9258 Results abstract; figures 2,7 ---	1,3-12, 14-25
X	WO 97 45439 A (STURLEY STEPHEN L ;UNIV COLUMBIA (US)) 4 December 1997 (1997-12-04) Sequence listing; claims abstract ---	1,3-12, 14-25
X	DATABASE EMBL [Online] Ac005917, 1 November 1998 (1998-11-01) LIN, X ET AL.: "Arabidopsis thalina chromosome 11 BAC F3P11 genomic sequence" XP002132392 see sequence ---	1,3-12, 14-25
X	DATABASE EMBL [Online] AC003058, 18 November 1997 (1997-11-18) ROUNSLEY.S.D ET AL.: "Arabidopsis thalina chromosome 11 BAC F27F23 genomic sequence, complete sequence" XP002132393 see sequence ---	1,3-12, 14-25
X	WESELAKE, RANDALL J. (1) ET AL: "Triacylglycerol biosynthesis and gene expression in microspore-derived cell suspension cultures of oilseed rape." JOURNAL OF EXPERIMENTAL BOTANY, (JAN., 1998) VOL. 49, NO. 318, PP. 33-39. , XP002132569 abstract ---	1,3-12, 14-25
X	LITTLE ET AL: "Solubilization and characterization of diacylglycerol acyltransferase from microspore-derived cltures of oilseed rape" BIOCHEMICAL JOURNAL,GB,PORTLAND PRESS, LONDON, no. 304, 1 January 1994 (1994-01-01), pages 951-958, XP002079126 ISSN: 0264-6021 cited in the application the whole document ---	1,3-12, 14-25
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## INTERNATIONAL SEARCH REPORT

International Application No

P US 99/28354

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PACHECO-MOISES F ET AL: "Regulation of acyltransferase activity in immature maize embryos by abscisic acid and the osmotic environment." PLANT PHYSIOLOGY, (1997 JUL) 114 (3) 1095-101. , XP002132305 the whole document	1,3-12, 14-25
A	--- CASES S ET AL: "Identification of a gene encoding an acyl CoA: diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,US,NATIONAL ACADEMY OF SCIENCE. WASHINGTON, vol. 95, no. 22, 27 October 1998 (1998-10-27), pages 13018-13023, XP002122745 ISSN: 0027-8424 cited in the application Disclosed is the Acyl CoA:diacylglycerolacyltransferase (DGAT, EC 2.3.1.20) catalyzing diacylglycerol, by using diacylglycerol and fatty acyl CoA. Expression of a mouse cDNA. abstract; figure 2	1,3-12, 14-25
A	--- FRENTZEN M (REPRINT): "Acyltransferases from basic science to modified seed oils" FETT - LIPID,DE,WILEY-VCH VERLAG,WEINHEIM, vol. 100, no. 4/05, May 1998 (1998-05), pages 161-166, XP002122744 ISSN: 0931-5985 abstract	1,3-12, 14-25
A	--- ICHIHARA K ET AL: "DIACYLGLYCEROL ACYLTRANSFERASE IN MATURING SAFFLOWER SEEDS: ITS INFLUENCES ON THE FATTY ACID COMPOSITION OF TRIACYLGLYCEROL" BBA - LIPIDS AND LIPID METABOLISM,NL,ELSEVIER SCIENCE BV. AMSTERDAM, vol. 958, no. 1, 19 January 1988 (1988-01-19), pages 125-129, XP000881625 ISSN: 0005-2760 abstract	1,3-12, 14-25
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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/28354

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>NYKIFORUK ET AL.: "Isolation and Characterization of a cDNA Encoding a Second Putative Diacylglycerol Acyltransferase"</p> <p>PLANT PHYSIOL.,</p> <p>vol. 121, no. 3, 1999, pages 1053-1053,</p> <p>XP002132391</p> <p>the whole document</p> <p>---</p>	1,3-12, 14-25
P,X	<p>HOBBS ET AL. : "Cloning of a cDNA encoding diacylglycerol acyltransferase from Arabidopsis thaliana and its functional expression"</p> <p>FEBS LETTERS,</p> <p>vol. 452, 11 June 1999 (1999-06-11), pages 145-149, XP002122747</p> <p>abstract</p> <p>figure 1</p> <p>---</p>	1,3-12, 14-25
P,X	<p>DATABASE EMBL [Online]</p> <p>Q9xGR5, 1 November 1999 (1999-11-01)</p> <p>NYKIFORUK C.L. ET AL. : "Putative Diacylglycerol Acyltransferase (EC 2.1.1.20)"</p> <p>XP002139487</p> <p>abstract</p> <p>---</p>	1,3-12, 15-22
P,X	<p>DATABASE EMBL [Online]</p> <p>Q9S7f2,</p> <p>ZOU,J. ET AL.: "The Arabidopsis thaliana TAG1 gene encodes for a diacylglycerol"</p> <p>XP002139488</p> <p>abstract</p> <p>---</p>	1,3-12, 15-25
P,X	<p>DATABASE EMBL [Online]</p> <p>AC: AJ238008, 18 June 1999 (1999-06-18)</p> <p>ZOU J.: "Arabidopsis thaliana mRNA for diacylglycerol acyltransferase"</p> <p>XP002139489</p> <p>abstract</p> <p>---</p>	1,3-12, 15-22
E	<p>WO 99 67403 A (UNIV CALIFORNIA)</p> <p>29 December 1999 (1999-12-29)</p> <p>the whole document</p> <p>---</p>	1,3-12, 14-25
E	<p>WO 99 63096 A (LASSNER MICHAEL W ; RUEZINSKY DIANE M (US); CALGENE LLC (US))</p> <p>9 December 1999 (1999-12-09)</p> <p>SEQ ID NO 2</p> <p>the whole document</p> <p>-----</p>	1,3-12, 15-25



JUL 05 2000

PATENT RECORDS  
CENTER

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To:  
E.I. DU PONT DE NEMOURS AND COMPANY  
Legal Patent Records Center  
Attn. FEULNER, GREGORY J.  
1007 Market Street  
Wilmington, Delaware 19898  
UNITED STATES OF AMERICA

NOTIFICATION OF TRANSMITTAL OF  
THE INTERNATIONAL SEARCH REPORT  
OR THE DECLARATION

(PCT Rule 44.1)

Date of mailing (day/month/year) 28/06/2000	
Applicant's or agent's file reference BB1295 PCT	<b>FOR FURTHER ACTION</b> See paragraphs 1 and 4 below
International application No. PCT/US 99/ 28354	International filing date (day/month/year) 01/12/1999
Applicant E.I. DU PONT DE NEMOURS AND COMPANY et al.	

1. ☒ The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

**Filing of amendments and statement under Article 19:**

The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

**When?** The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

**Where?** Directly to the International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland  
Facsimile No.: (41-22) 740.14.35

**For more detailed instructions,** see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. ☐ **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.


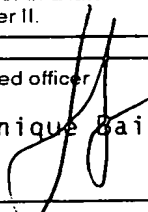
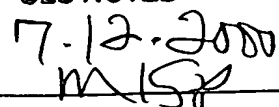
☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90bis.1 and 90bis.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority  European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Véronique Baillou 	<b>CLS NOTED</b> 7.12.2000 
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## NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

### INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

#### What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

#### When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

#### Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

#### How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

**The amendments must be made in the language in which the international application is to be published.**

#### What documents must/may accompany the amendments?

##### Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

**The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.**

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

- (i) the claim is unchanged;
- (ii) the claim is cancelled;
- (iii) the claim is new;
- (iv) the claim replaces one or more claims as filed;
- (v) the claim is the result of the division of a claim as filed.

**The following examples illustrate the manner in which amendments must be explained in the accompanying letter:**

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:  
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:  
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:  
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or  
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:  
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

**"Statement under article 19(1)" (Rule 46.4)**

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

**It must be in the language in which the international application is to be published.**

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

**Consequence if a demand for international preliminary examination has already been filed**

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

**Consequence with regard to translation of the international application for entry into the national phase**

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

# PATENT COOPERATION TREATY

RECEIVED

From the  
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

MAR 14 2001

To:

Andrew L. Schaeffer  
E.I. DU PONT DE NEMOURS AND COMPANY  
Legal Patent Records Center  
1007 Market Street  
Wilmington, Delaware 19898  
ETATS-UNIS D'AMERIQUE

KL

PATENT RECORDS  
NOTIFICATION OF TRANSMITTAL  
OF THE INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT  
(PCT Rule 71.1)

Date of mailing  
(day/month/year) 06.03.2001

Applicant's or agent's file reference  
BB1295 PCT

## IMPORTANT NOTIFICATION

International application No.  
PCT/US99/28354

International filing date (day/month/year)  
01/12/1999

Priority date (day/month/year)  
02/12/1998

Applicant  
E.I. DU PONT DE NEMOURS AND COMPANY et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

#### 4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/

 European Patent Office  
D-80298 Munich  
Tel. +49 89 2399 - 0 Tx: 523656 epmu d  
Fax: +49 89 2399 - 4465

Authorized officer

Guerin, A

Tel. +49 89 2399-8061

REY NOTED



02 JE 2001

REC'D 09 MAR 2001

WIPO

PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference BB1295 PCT	<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/US99/28354	International filing date (day/month/year) 01/12/1999	Priority date (day/month/year) 02/12/1998
International Patent Classification (IPC) or national classification and IPC C12N9/00		
Applicant E.I. DU PONT DE NEMOURS AND COMPANY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.



2. This REPORT consists of a total of 8 sheets, including this cover sheet.

- ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☒ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 26/06/2000	Date of completion of this report 06.03.2001
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Meyer, W Telephone No. +49 89 2399 8157 

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/28354

## I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).):*

### Description, pages:

1-29 as originally filed

### Claims, No.:

1-25 as originally filed

### Drawings, sheets:

1/3-3/3 as originally filed

### Sequence listing part of the description, pages:

1-25, filed with the letter of 13.01.00

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/28354

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

### III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
- ☒ claims Nos. 2 and 13.

because:

- ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
- ☒ no international search report has been established for the said claims Nos. 2 and 13.

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

- ☐ the written form has not been furnished or does not comply with the standard.
- ☐ the computer readable form has not been furnished or does not comply with the standard.

### IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/28354

- ☐ restricted the claims.
- ☐ paid additional fees.
- ☐ paid additional fees under protest.
- ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is
- ☐ complied with.
  - ☒ not complied with for the following reasons:  
**see separate sheet**
4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:
- ☐ all parts.
  - ☒ the parts relating to claims Nos. 3 and 14 (completely), 1, 4-11 and 15-25 (limited to SEQ ID NO 1, 2 and functional variants thereof).

## V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

### 1. Statement

Novelty (N)	Yes:	Claims	4-11, 13 and 15-25 (limited to SEQ ID NO 1, 2 and functional variants thereof)
	No:	Claims	1, 3 and 14 (limited to SEQ ID NO 1, 2 and functional variants thereof)
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1, 3-11, 13-25 (limited to SEQ ID NO 1, 2 and functional variants thereof)
Industrial applicability (IA)	Yes:	Claims	1, 3-11, 13-25 (limited to SEQ ID NO 1, 2 and functional variants thereof)
	No:	Claims	

2. Citations and explanations  
**see separate sheet**

## VI. Certain documents cited



**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/US99/28354

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1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

**see separate sheet**

**Re Item I**

1. The Applicant has been invited to restrict or to pay additional fees. The Applicant did not restrict nor pay additional fees. As the "putative" inventions appear to be of equivalent importance, the application will be prosecuted on the basis of the first mentioned invention, in other words the invention first mentioned in the International Search Report: The subject-matter of **3, 14 (completely) and 1, 4-11 and 15-25 (all partially)** relating to SEQ ID NO. 1, 2 and functional variants thereof.
2. The Applicant should limit the application to the invention examined and excise those parts of the application relating to other inventions.

**Re Item V**

3. As the priority document for the application was not available at the time of drafting of the present written opinion, it is established under the assumption that the entire subject-matter is entitled to the claimed priority. If this is not the case, the 'P' documents cited in the search report may have to be considered for novelty and/or inventive step. These documents would be detrimental to most, if not all, of the claimed subject-matter (Article 33(2), (3) PCT).
4. The documents mentioned in this written opinion are numbered as in the International Search Report (ISR), i.e. D1 corresponds to the first document of the ISR etc.
5. The subject-matter of **claims 1, 3 and 14** in their limitation to SEQ ID NO. 1, 2 and functional variants thereof is not new in the sense of Article 33(2) PCT. The subject-matter is anticipated by D1 (D1, Abstract). This document discloses the sequence of a putative acyl-CoA Cholesterol Acyltransferase, which has 97.4% identify in 228 amino acids overlap. Thus D1 a sequence having at least 50 amino acids which has at least 80% identity to SEQ ID NO. 2.
6. The subject-matter of **claims 5-11, 13 and 15-25** in its limitation to SEQ ID NO. 1,



2 and functional variants thereof is referring back or dependent on the subject-matter of present **claims 1, 3 or 14**. The subject-matter of **claims 5-11, 13 and 15-25** does not appear to include any additional matter which would render them inventive as such. These claims appear to be concerned with mere technical variations of the known subject-matter of **claim 1, 3 or 14**. Consequently, they do not fulfil the requirements of Article 33(3) PCT.

7. The subject-matter of present **claim 4** in its limitation to SEQ ID NO. 1 or 2, fulfills the requirement of Article 33(2) PCT.

8. However, the subject-matter of **claim 4** does not involve an inventive step (Article 33(3) PCT): D1 is considered to represent the closest prior art and discloses a sequence that has 97.4% identify with SEQ ID NO. 2.

The difference between D1 and the subject matter of **claim 4** of the present application is the provision of polynucleotide consisting of SEQ ID NO. 1 or coding for a polypeptide consisting of SEQ ID NO. 2.

Starting from D1, the underlying technical problem is to randomly mutate the sequence of D1.

The subject-matter of the claims referred to above is considered to solve this problem. However, the solution proposed in **claim 4** of the present application cannot be considered as involving an inventive step (Article 33(3) PCT) for the following reasons:

Once a DNA is available the a skilled person would without the need of an inventive skill change the sequence of the given sequence and arrive to the claimed sequence. Consequently, **claim 4** does not fulfil the requirements of Article 33(3) PCT.

9. The applicant's attention is further drawn to Documents D2-D7. It appears that these documents are also prejudicial to the novelty and/or the inventive activity of the subject-matter of **claims 1, 3-12 and 15-25**:

D2 discloses the characterization of two human genes encoding acyl coenzyme A: cholesterol Acyltransferase-related enzymes in that has 61% identify over a stretch of 49 amino acids to SEQ ID NO. 1

D3 discloses a human Cholesterol Acyltransferase II, or ACAT related gene product 1( ARGP 1) that has 41.9% identity in 399 amino acids overlap with SEQ

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ID NO 2.

D4 discloses a sequence that is 100% identical to SEQ ID NO. 1 in 580 bp overlap.

D5 discloses a sequence that is 100% identical to SEQ ID NO. 1 in 520 bp overlap.

D6-D8 disclose the diacylglycerol transferases activity in different plant species and how to clone plant derived cDNAs of triacylglycerol biosynthetic enzymes and associated proteins.

**Re Item VI**

Application No Patent No	Publication date (day/month/year)	Filing date (day/month/year)	Priority date (valid claim) (day/month/year)
WO 99/63096	12.09.1999	04.06.1999	05.06.1998
WO 99/67403	29.12.1999	28.8.1998	24.06.1998

10. When considering to file this application at the EPO, the applicants' attention is drawn to WO 99/63096 and WO 99/67403 published on 12.09.1999 or 29.12.1999, respectively. Their content as filed is considered, pursuant to Article 54(3) and (4) EPC, as comprised in the state of the art and is prejudicial to the novelty of the subject-matter to the claims of the present application insofar as the same Designated Contracting States are concerned.